Bone Matrix Constituents Stimulate Interleukin-1 Release from Human Blood Mononuclear Cells

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Abstract

To test the hypothesis that mononuclear cells are stimulated to release interleukin 1 (IL-1) by bone fragments released in the bone microenvironment during the remodeling cycle, we have investigated the effects of bone matrix and some of its constituents on IL-1 secretion from peripheral blood mononuclear cells (PBMC). Increases in IL-1 activity were observed when either PBMC or adherent monocytes, but not lymphocytes depleted of monocytes, were co-cultured with either human or rat bone particles but not with latex particles of similar size. Co-culture of PBMC with bone particles in a transwell system where the cells were physically separated from the bone particles, or with osteoblast- or osteoclast-covered bone particles, did not stimulate IL-1 release, indicating that a physical contact between PBMC and the bone surface is required for eliciting IL-1 release. This was confirmed by the finding of a lower stimulatory effect of bone particles pretreated with etidronate, a bisphosphonate which decreases the bone binding capacity of PBMC. Constituents of bone matrix, such as collagen fragments, hydroxyproline, and, to a lesser extent, transforming growth factor-β, but not osteocalcin, α2HS glycoprotein, fragments of either bone sialoprotein or osteopontin, and fibronectin, stimulated PBMC IL-1 release in a dose-dependent fashion. Collagen-stimulated IL-1 release was partially and specifically inhibited by a monoclonal antibody directed against the α1β1 integrin cell surface collagen receptor. These data demonstrate that products of bone resorption, known to be chemotactic for mononuclear cells, stimulate PBMC IL-1 activity. These findings may help explain previous documentation of increased IL-1 secretion by circulating monocytes obtained from patients with high turnover osteoporosis. (J. Clin. Invest. 1991, 87:221–228). Key words: bone matrix • collagen • integrin • interleukin 1 • mononuclear cells • osteoporosis

Introduction

Interleukin 1 (IL-1) is a family of several, closely related, low molecular weight proteins produced by many types of mammalian cells, including bone cells (1–3). Although best known for its ability to promote lymphocyte proliferation (4, 5) and for its involvement in inflammation and wound healing (6), IL-1 is also recognized for its multiple and important effects on bone. In fact, not only is IL-1 a powerful stimulator of bone resorption in vitro (7–9) and in vivo (10, 11), but it modulates bone cell proliferation (12–15) and collagen and noncollagen bone protein synthesis (16, 17), and induces the secretion of other bone active cytokines from bone cells or marrow resident mononuclear cells (18, 19).

In previous studies (20), we reported that circulating monocytes from subjects with “high turnover” osteoporosis, a condition marked by increased bone resorption and bone formation, elaborated higher amounts of IL-1 in vitro than those from subjects with “low turnover” osteoporosis, a disorder in which bone formation is impaired. More recently, we demonstrated that women in the early menopausal period, when enhanced bone resorption causes rapid bone loss, also display increased monocyte IL-1 activity that is blocked by ovarian steroid therapy (21). Although these observations implied a causative role for IL-1 in postmenopausal bone loss, they did not offer any insight into the mechanism(s) that lead(s) to IL-1 release in states of high bone turnover.

Monocytes are often found adjacent to bone resorbing surfaces, and resorbing bone and bone peptides released in the local microenvironment during bone resorption have been shown to be chemotactic for monocytes (22–25). Among these peptides are collagen, osteocalcin, α2HS glycoprotein, and transforming growth factor-β (TGF-β), a cytokine produced by bone cells and enclosed in the bone matrix (25) that stimulates the expression of IL-1 messenger RNA in monocytes (24). Release of bone matrix digestion products and/or osteoblast secretory products might account for the local recruitment and activation of mononuclear cells and the resulting release of IL-1. Secretion of IL-1 from intraskeletal mononuclear cells could, in turn, play a fundamental role in the regulation of bone remodeling. Similarly, the bone-sparing effect of ovarian steroids could be explained by a regulatory effect of these hormones on the capacity of mononuclear cells, or perhaps their precursors, to release IL-1 in response to exposure to bone matrix constituents. In this study, we examined bone matrix and some of its constituents for their effects on IL-1 release from human peripheral blood mononuclear cells (PBMC). We report that adherence of PBMC to bone matrix markedly increases the secretion of IL-1 from PBMC into the culture me-

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Received for publication 29 March 1990 and in revised form 20 July 1990.

J. Clin. Invest. © The American Society for Clinical Investigation, Inc. 0021-9738/91/01/0221/08 $2.00 Volume 87, January 1991, 221–228

1. Abbreviations used in this paper: EHD P, 1-hydroxy-1,1-ethane diphosphate (etidronate); LAP, lipoprotein A-associated protein; PB, polymixin B; TGF-β, transforming growth factor-β.
The data also reveal that the increase in IL-1 activity can be induced by some, but not all, well-characterized bone matrix constituents.

**Methods**

Unless otherwise specified, reagents and media were from the Sigma Chemical Co., St. Louis, MO.

**Mononuclear cell cultures.** PBMC cultures were prepared from healthy volunteers as described (20, 21). Briefly, freshly drawn blood was fractionated on Ficol/Hypaque, and the PBMC were removed from the interface and washed twice with RPMI 1640 medium. The cells were resuspended in complete medium (RPMI 1640 medium supplemented with 5% [vol/vol] heat-inactivated fetal bovine serum [defined, Sterile Systems, Logan, UT; endotoxin, 0.038 ng/ml] at a concentration of 1 × 10^6 cells/ml, and 1-ml aliquots incubated in 16-mm wells of 24-well tissue culture plates for 48 h at 37°C in a humidified atmosphere of 5% CO_2/95% air. For some experiments 1-ml aliquots were allowed to adhere for 2 h at 37°C. After incubation the nonadherent cells (enriched lymphocytes) were removed from the wells, resuspended in 1 ml of medium and incubated for 48 h. The adherent population (enriched monocytes) was washed twice with RPMI 1640 to remove any remaining nonadherent cells. The adherent cells were then incubated in 1 ml of complete medium for 48 h. The adherent population was subsequently stained for the monocyte/macrophage-specific enzyme α-naphthyl acetate esterase and was found to be > 95% monocytes. Monocytes composed 19.5±4.1% of the entire original mononuclear population isolated by Ficol density gradient centrifugation. In all experiments, at the end of the 48-h incubation, conditioned media were collected and assayed for endotoxin by the chromogenic Limulus amebocyte lysate assay (Whittaker M.A. Bioproducts, Walkersville, MD). Endotoxin was not detected at the level of sensitivity of the assay (≥ 10 pg/ml). The media were then passed through 0.22-μm filters and stored at −20°C until assayed for IL-1.

**IL-1 assay.** The PBMC-conditioned media were assayed for IL-1 activity (IL-1α and IL-1β) by assessing the increase in mitogen-induced proliferation of the helper T cell D10.G4.1 (D10 cells) as previously described (20, 21, 26). The IL-1 standard used in the assays was ultrapure IL-1 (Genzyme, Boston, MA), except for the IL-1 used in the neutralization assay, which was recombinant IL-1α or IL-1β (Genzyme). The IL-1 activity, measured by quantitating the thymidine incorporation in the D10 cells, was converted to units per milliliter by performing a log-logit transformation of the serial dilution curves and determining the dilution of the test sample that yielded a value corresponding to 50% of the standard IL-1 maximum activity. The standard IL-1 activity was arbitrarily set at 100 U/ml. The test sample units were then determined as follows: Activity (sample) = 2^−a+b × 100 U/ml (standard IL-1), where a is the dilution of the test sample yielding 50% of the standard IL-1 activity and b is the dilution of the standard IL-1 yielding 50% maximal activity.

The inter assay and the intras assay variability of this assay was 29% and 10%, respectively. Recovery of added recombinant IL-1β was ≥ 90. The minimum amount of lipopolysaccharide (LPS) capable of stimulating IL-1 secretion was 10–100 pg/ml.

Since PBMC may secrete agents in addition to IL-1 that are comitogenic in the T cell assay (27–29), we verified our findings as indicative of the presence of IL-1 by demonstrating inhibition of the PBMC-conditioned media effect in the presence of monoclonal anti-IL-1α and anti-IL-1β antibodies (kindly provided by John Kenney, Syntex Corp., Palo Alto, CA). These neutralization experiments were performed by incubating serial dilutions of the PBMC culture medium for 2 h with anti-IL-1α (1:20 dilution), anti-IL-1β (1:200 dilution), or control serum at 37°C before assay of the D10 cells.

**Preparation of test materials.** Intact type I collagen was extracted from rat tails according to the method of Piez et al. (30). Heat-denatured type I guinea pig skin collagen was prepared as previously described (31). Denatured type I collagen peptides were produced by digesting heat-denatured guinea pig skin collagen with purified rat uterus collagenase as previously described (31). Human α2HS glycoprotein (49,000 mol wt) was isolated from human serum as previously described (25). Purified bovine osteocalcin (BGP), extracted from lyophilized bone with EDTA and purified by gel filtration as described by Price et al. (32), was kindly provided by Dr. P. Price (University of California, San Diego). Purified bovine TGF-β was kindly provided by Dr. Saeid Seyedin (Collagen Corp., Palo Alto, CA). Purified bovine fibronectin was kindly provided by Dr. John A. McDonald (Washington University School of Medicine, St. Louis, MO). Adherence promoting Arg-Gly-Asp (RGD)-containing fragments of synthetic bone sialoprotein (18 aminoacids) and osteopontin (20 aminoacids) were kindly provided by Dr. Pamela Robey (National Institutes of Health, Bethesda, MD). Commercial 50-μm hydroxyapatite crystals were washed extensively, and suspended at 10 mg/ml in pyrogen-free phosphate-buffered saline (PBS).

For some experiments, hydroxyapatite and heat-denatured collagen were hydrolyzed with 0.01 N NaOH in 90% ethanol at 37°C for 60 min, with frequent mixing (33). The reaction was arrested by neutralizing with acetic acid and reducing the sample temperature to 4°C. Control samples were incubated in 90% ethanol without NaOH. Collagen was resuspended and neutralized by dialysis against acetic acid, phosphate buffer, and normal saline.

All test materials contained < 10 pg/ml endotoxin in the Limulus amebocyte lysate assay (data not shown).

**Bone particle preparation.** Human and rat devitalized bone particles were prepared as previously described by Teitelbaum et al. (34). Briefly, human ribs or rat long bones were dissected free of periosteum and soft tissue, split to expose the marrow cavity, washed extensively with sterile 0.9% NaCl, and air-dried for 1 wk at 45°C. The bones were then ground in a mill (Spex Industries, Inc., Metuchen, NJ) and particles < 50 μm in diameter were collected by passing the coarse powder through a screen (Cistron Corp., Lebanon, PA). These particles were then sterilized by ultraviolet irradiation. Nondevitalized human and rat bone particles were prepared in a similar manner by using sterile equipment and by omitting the air drying and the ultraviolet irradiation steps. Osteoblast-covered human and rat bone particles were prepared by maintaining the live bone particles in culture for 7–10 d. Under these conditions, cells gradually grew on the surface of the particles covering the entire matrix surface. These cells were characterized as osteoblasts because they were intensively positive for alkaline phosphatase and Van Kossa staining and, when isolated by collagenase digestion, secreted cAMP in response to PTH (data not shown). Chicken osteoblast-covered bone particles were prepared by incubating devitalized rat bone particles with chicken osteoclasts, isolated according to the methods of Zambonin-Zallone et al. (35) and Blair et al. (36) for 18 h at 37°C. The bone particles and attached osteoclasts were then separated from unbound cells by unit gravity sedimentation in PBS for 10 min at 27°C. In some experiments, bone particles were preincubated with 1-hydroxy-1, 1 ethane diphosphinate (EHDP), or etidronate (Norwich Eaton Pharmaceuticals, Inc., Norwich, NY), 80 μmol for 24 h at 37°C. The EHDP-treated bone particles were then pelleted by centrifugation and washed twice with fresh medium. In all the experiments carried out using PBMC cultures or lymphocyte-enriched preparations, the tissue culture plates were coated with a thin layer of the bone particle of choice. The cells were then added and cultured for 48 h. Similar results were obtained whether test cells were added before or after bone particles (data not shown). In the experiments with monocyte-enriched preparations, the same amount of bone particles was added to the adherent monocytes after the removal of the non-adherent mononuclear cells. The optimal concentration of bone particles (1 mg/ml) was determined before the study. Increasing the bone particle concentration as much as 10-fold did not alter the results. However, decreasing the amount of bone particles so that about ≥ 30% of the culture well surfaces were not covered decreased the mononuclear cell response.

**Antibodies.** The P1H5 monoclonal antibody directed against the α2β1 integrin cell surface collagen receptor was generously provided by
IL-1 activity in the culture medium of PBMC co-cultured with either rat or human bone particles. IL-1 activity in the culture media of unstimulated PBMC (controls) was 2.1±0.9 U/ml. To allow comparisons of data from different experiments, results are shown as treated/control (T/C) ratio. Abbreviations: OB, osteoblasts; OC, osteoclasts; EHDP, etidronate. "Transwell" refers to experiments carried out in a culture system where PBMC were separated from the bone particles by a membrane permeable to the culture medium only. *P < 0.05; **P < 0.01; ***P < 0.001 compared to controls.

Figure 1. IL-1 activity in the culture medium of PBMC co-cultured with either rat or human bone particles. IL-1 activity in the culture media of unstimulated PBMC (controls) was 2.1±0.9 U/ml. To allow comparisons of data from different experiments, results are shown as treated/control (T/C) ratio. Abbreviations: OB, osteoblasts; OC, osteoclasts; EHDP, etidronate. "Transwell" refers to experiments carried out in a culture system where PBMC were separated from the bone particles by a membrane permeable to the culture medium only. *P < 0.05; **P < 0.01; ***P < 0.001 compared to controls.

Results

Bone particles stimulate the secretion of IL-1 from normal human PBMC. In a set of 25 experiments (Fig. 1), IL-1 activity in the culture media of unstimulated PBMC incubated in plastic culture wells for 48 h was 2.1±0.9 U/ml (mean±SEM) (median 0.39, range 0.1–16.4).

A similar amount of IL-1 was released into the medium by PBMC cultured over a layer of ~ 50-μm latex particles (Fig. 1). In this condition PBMC adhered strongly to but did not phagocytize the latex particles. PBMC co-cultured with similar amounts of devitalized or live rat or human bone particles adhered to the bone particles and released significantly higher amounts of IL-1 in the culture media. Devitalized human particles were more potent in inducing IL-1 activity than devitalized rat particles (25.0- vs. 11.1-fold increase, respectively). However, the highest IL-1 activities were found in the media of PBMC co-cultured with live rat (43-fold increase) and human (49-fold increase) bone particles, suggesting that soluble factors released by osteocytes within the bone particles contribute to the enhanced IL-1 activity.

In order to determine whether a physical contact between PBMC and bone surface is required to activate IL-1 release, PBMC were co-cultured with osteoblast-covered human or rat particles or osteoclast-covered rat bone particles. Under these conditions, PBMC were in close contact with the bone cells covering the particles, but were separated from the matrix surface itself. In each of these experiments, IL-1 activity was similar to that in control PBMC cultures, suggesting that IL-1 secretion requires the physical contact of PBMC to specific constituents of the bone surface. Conditioned media from osteoblast- or osteoclast-covered bone cultures did not demonstrate a decreased PBMC response to uncovered bone particles nor a diminished ability of recombinant IL-1β to stimulate D10 cells proliferation (data not shown). These findings suggest that cells covering the bone particles did not secrete factor(s) that inhibit PBMC IL-1 release.

To confirm that physical contact of PBMC and bone is required for inducing the PBMC response to bone, experiments were carried out with a transwell culture system in which PBMC and devitalized rat bone particles were incubated in two chambers divided by a membrane (pore size 0.4 mm) permeable to the culture media, but which prohibited cell-bone contact. Under these conditions, the IL-1 activity in the transwell cultures was similar to the activity in control PBMC cultures.

To assess whether interferences with the attachment of PBMC to bone surface affect IL-1 release, devitalized rat bone particles were pretreated for 48 h with EHDP, a substance that decreases the binding of PBMC and osteoclasts to the bone surface (39, 40). As shown in Fig. 1, pretreatment with EHDP (80 μmol) significantly decreased the ability of rat bone particles to stimulate IL-1 release. To investigate whether a toxic effect of EHDP on PBMC may have accounted for those findings, phytohemagglutinin (PHA)-stimulated PBMC were incubated with concentrations of EHDP (Table I) up to fivefold higher than those used to pretreat the bone particles. This treatment resulted in no significant change in the amount of IL-1 released into the culture media. Similarly, EHDP did not decrease the ability of guinea pig type I collagen (see below and Table I) to increase IL-1 activity from unstimulated PBMC.

To determine whether bone particles induce IL-1 release from monocytes, lymphocytes, or both, devitalized rat bone particles were co-cultured with adherent monocytes or lymphocytes depleted of monocytes. In each of five experiments (Table II), monocytes but not lymphocytes adhered to the bone particles and released increased amounts of IL-1.

Effect of bone matrix constituents on PBMC IL-1 activity. To investigate the nature of the bone constituent(s) accounting for the release of IL-1, PBMC were incubated with various

<table>
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<th>Stimulants</th>
<th>EHDP</th>
<th>PHA</th>
<th>Type I collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td>μM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>3.9±0.8</td>
<td>4,364±426</td>
<td>3,628±946</td>
</tr>
<tr>
<td>PHA</td>
<td>3.1±0.9</td>
<td>3,911±398</td>
<td>3,712±1,001</td>
</tr>
<tr>
<td>25</td>
<td>4.2±0.7</td>
<td>4,469±422</td>
<td>3,401±880</td>
</tr>
<tr>
<td>50</td>
<td>3.6±0.9</td>
<td>4,304±480</td>
<td>3,395±972</td>
</tr>
<tr>
<td>100</td>
<td>3.2±0.6</td>
<td>4,732±401</td>
<td>3,983±1,057</td>
</tr>
<tr>
<td>200</td>
<td>4.1±0.8</td>
<td>3,913±422</td>
<td>3,661±1,112</td>
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</tbody>
</table>

PBMC were incubated with EHDP and either PHA or guinea pig type I collagen for 48 h. The culture medium was then assayed for IL-1. Data are reported as mean±SEM.
purified bone substances. In a first set of experiments, PBMC were cultured in a well coated with a gel of intact rat tail type I collagen or a layer of hydroxyapatite crystals. Assays of the resulting conditioned media revealed an increase in IL-1 activity with both substances (Table III). However, the response elicited by collagen was higher than the one induced by hydroxyapatite. Preincubation of hydroxyapatite with EHDP (80 μmol) reduced by 65% the stimulatory effect on IL-1 activity, further indicating that EHDP may prevent IL-1 release by a specific physicochemical interaction with PBMC and hydroxyapatite.

In a second set of experiments, dose-response curves were generated by incubating PBMC with one of the following: a soluble preparation of heat-denatured guinea pig type I skin collagen or type I collagen fragments produced by the action of rat uterus collagenase on heat-denatured collagen (Fig. 2, left), hydroxyapatite crystals, TGF-β, α2HS glycoprotein, osteocalcin, fibronectin, bone sialoprotein fragments, or osteopontin fragments. Guinea pig type I denatured collagen and collagen fragments both induced a significant (P < 0.001) increase in the amount of IL-1 released into the culture media. As shown in Fig. 2 (right), the magnitude of the response was dose dependent within the range tested (0.5–150 μg/ml). However, the collagen fragments produced by either a 2- or a 4-h digestion with mammalian collagenase were even more potent (P < 0.05) than intact collagen in inducing IL-1 release. Conversely, the amount of IL-1 released into the culture media after incubation with either 24- or 48-h digested fragments was lower than that elicited with intact collagen (Fig. 2, right). When PBMC were incubated with hydroxyapatite (Fig. 3), a dose-dependent stimulatory effect was also observed. However, the maximal response elicited by hydroxyapatite was significantly lower (P < 0.05) than that induced by either heat-denatured type I collagen or its fragments.

To assess whether collagen and hydroxyapatite induce the secretion of IL-1α, IL-1β, or both, PBMC culture media were incubated with monoclonal antibodies against human IL-1α and IL-1β. The results (Table IV) showed an almost complete inhibition of biological activity with the anti–IL-1β antibody and a partial reduction with the anti–IL-1α antibody, indicating that IL-1β was indeed responsible for the majority of the D10 cell proliferation activity.

Incubation of PBMC with purified bovine TGF-β1 induced a significant but much less substantial increase in IL-1 activity (Fig. 3). This response was dose dependent between 1 and 10 ng/ml, optimal at 10 pg/ml, and was abolished by boiling the TGF-β preparation for 10 min. Conversely, IL-1 activity was not stimulated by osteocalcin (0.1 ng/ml to 1 μg/ml), α2HS glycoprotein (10–12 to 10–3 M), fibronectin (0.1–100 μg/ml), bone sialoprotein fragments (0.1–100 μg/ml), or osteopontin fragments (0.1–100 μg/ml). The effect of fibronectin, osteopontin, and bone sialoprotein on IL-1 release was also investigated by culturing PBMC in wells coated with each of these agents. The dose responses obtained with these experiments were similar to those obtained by dissolving the test substances in PBMC cultures (data not shown).

A monoclonal antibody which recognizes the αβ1 integrin receptor blocks the collagen-induced increase in IL-1 activity. To determine whether a specific receptor mediates the IL-1 response to collagen, antibody neutralization experiments were performed. As shown in Fig. 4, the P1H5 monoclonal antibody directed against the αβ1-integrin, a cell surface collagen receptor, significantly decreased the IL-1 release in response to heat-denatured collagen but not to both hydroxyapatite and LPS. The spontaneous release of IL-1 from unstimulated PBMC was also not affected by the P1H5 antibody. These findings suggest that the IL-1 release induced by collagen is mediated, at least in part, by the αβ1-integrin. The control antibody, 10E5, had no effect on the IL-1 released in response to collagen.

To investigate the contribution of the αβ1 receptor to the release of IL-1 induced by the adherence of PBMC to bone particles, PBMC were co-cultured with devitalized bone particles and either the P1H5 or the 10E5 antibody. The antibody P1H5 decreased, although not significantly, the IL-1 release induced by rat bone particles, suggesting that interaction with the αβ1-integrin receptor is one, but not the exclusive, mechanism by which bone particles stimulate PBMC IL-1 release.

Polymyxin B and alkaline hydrolysis do not abolish the PBMC response to collagen and hydroxyapatite. LPS is one of the most potent stimulants of IL-1 release from PBMC (41). To rule out the possibility that contamination with levels of LPS below the limit of detection of the Limulus amebocyte lysate assay (< 10 pg/ml) might have accounted for the IL-1 stimulatory activity observed with our collagen and hydroxyapatite preparations, we cultured PBMC with these bone constituents and polymyxin B (PB). PB is a polypeptide antibiotic which, at low concentrations, blocks many effects of LPS on immune cells including IL-1 release (42). Although at high concentrations PB may directly stimulate IL-1 secretion (43), concentrations ≤ 0.5 μg/ml effectively block levels of LPS ≤ 1 ng/ml.

Table II. Effect of Devitalized Rat Bone Particles on IL-1 Activity from Peripheral Blood Monocytes, Lymphocytes, and Total Mononuclear Cells (PBMC)

<table>
<thead>
<tr>
<th>Group</th>
<th>IL-1 activity</th>
<th>Treated/control IL-1 activity</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>U/ml</td>
<td></td>
</tr>
<tr>
<td>Unstimulated cells</td>
<td>0.1±0.1</td>
<td>0.1±0.1</td>
</tr>
<tr>
<td>Cells + rat bone particles</td>
<td>56.7±9.5*</td>
<td>1.9±1.4</td>
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</table>

PBMC were incubated in culture wells coated with a collagen gel of rat tail type I collagen or a layer of hydroxyapatite crystals for 48 h. The culture medium was then assayed for IL-1. Data are reported as mean±SEM.

* P < 0.001; † P < 0.01, compared to controls.

Table III. Effect of Rat Tail Type I Collagen, Hydroxyapatite, and EHDP-treated Hydroxyapatite on PBMC IL-1 Activity

<table>
<thead>
<tr>
<th>Group</th>
<th>IL-1 activity</th>
<th>Treated/control IL-1 activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U/ml</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>1.1±0.2</td>
<td></td>
</tr>
<tr>
<td>Collagen</td>
<td>4,964.2±359.6*</td>
<td>4,513</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>1,175.3±281.2†</td>
<td>1,068</td>
</tr>
<tr>
<td>EHDP-hydroxyapatite</td>
<td>411.2±98.6†</td>
<td>374</td>
</tr>
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</table>

PBMC were incubated in culture wells coated with a collagen gel of rat tail type I collagen or a layer of hydroxyapatite crystals for 48 h. The culture medium was then assayed for IL-1. Data are reported as mean±SEM.

* P < 0.001; † P < 0.01, compared to controls.
significant (P < 0.05). The PBMC response to hydroxyapatite was significant at P < 0.05 for concentrations ≥ 0.25 mg/ml and at P < 0.0001 for concentrations ≥ 1 mg/ml. The PBMC response to TGFB was significant (P < 0.05) at 0.01 ng/ml and was abolished by boiling for 10 min (data not shown).

IL-1 activity (data not shown). Similarly, the presence of bound LPS was excluded by the inability of hydroxyapatite and collagen preparations to decrease the PBMC response to added LPS (data not shown).

**Discussion**

In this study, we have shown that attachment of PBMC or adherent monocytes to the bone matrix surface is associated with a marked stimulation of IL-1 release. The data also indic...
were in physical contact with rat osteoblasts or chicken osteoclasts but could not adhere to the bone matrix surface. The results of the experiments with test substances treated with PB, alkaline hydrolysis, or boiling, and the inability of the suspension fluids to stimulate PBMC IL-1 release suggest that contamination with low levels of LPS or LAP is an unlikely explanation for our findings.

Stimulation of IL-1 activity appeared to be the result of a specific PBMC–bone surface interaction. Increased IL-1 activity was not observed, in fact, when PBMC were co-cultured with latex particles, to which cells strongly adhere. Although the intimate nature of this interaction remains unknown, attachment of the cells to the bone surface appears to be an essential step. Nonadherent monocyte-depleted lymphocytes were not stimulated to release IL-1 and IL-1 secretion was not increased when bone surfaces and mononuclear cells were not in physical contact. Similarly, co-culture of PBMC with bone particles which had been pretreated with EHDP, an agent which reduces the bone binding capacity of both PBMC (39) and osteoclasts (40), decreased but did not abolish the PBMC response to bone. As previously observed in similar studies (46), the EHDP inhibition of IL-1 release was not the result of a toxic effect on PBMC, as indicated by the inability of EHDP to inhibit the secretion of IL-1 from cells stimulated with PHA or incubated with collagen.

Previous studies have shown that the attachment of monocytes to bone is analogous to that of osteoclasts and depends on the constituents of the bone matrix (47). Our findings support these concepts and indicate that not only adherence, but also the secretory activity of PBMC is regulated by specific matrix constituents. Two of these which we found to have high stimulatory activity were collagen fragments, the most abundant bone peptide, and hydroxyapatite, the mineral constituent of bone. Whether specific receptors mediate the interaction of PBMC with hydroxyapatite has yet to be determined. However, the ability of a specific monoclonal antibody, P1H5, to decrease the PBMC response to collagen suggests that the interaction between collagen and these cells is mediated, at least in part, by a specific collagen receptor, the αβ1-integrin (48–50). This class of collagen receptor is indeed expressed on monocytes (49) and osteoclasts (51) but not on circulating lymphocytes (49). Moreover, the fact that products of short-term, but not long-term, collagenase digestion were more potent stimulators of IL-1 activity than undigested material suggests that specific collagen domains were first exposed and then partially digested as the length of the collagenase digestion increased.

Two other bone constituents that possess chemotactic properties, α2HS glycoprotein and osteocalcin (23), failed to stimulate IL-1 activity. Similarly, IL-1 activity was not stimulated by fibronectin, a ubiquitous adherence protein (52), bone sialoprotein, or osteopontin fragments. Adherence proteins containing the Arg-Gly-Asp (RGD) sequence (52, 53), a trait recognized by some but not all integrin receptors (48, 49) and osteocalcin have been hypothesized to participate in cell attachment to mineralized matrix because they bind tightly to collagen (52) and hydroxyapatite (54) and because vitamin-K depletion prevents cell adherence to bone (55). Our data do not negate this possibility; rather they suggest important functional differences between the bone fragments produced with bone resorption. Substances such as adherence proteins and osteocalcin may, in fact, play a role in regulating the homing and the attachment of mononuclear cells to bone, whereas others, such as collagen...
fragments, may be particularly important for regulating the secretion of bone resorbing agents such as IL-1.

Although the regulation of bone remodeling is still conjectural at best, several lines of evidence suggest a role for IL-1 in either initiating or modulating this remodeling phenomenon. Recent studies in rodents have shown that IL-1 stimulates bone turnover both locally and systemically, causing bone loss and hypercalcemia (10, 11). Increased secretion of IL-1 from mononuclear cells has been reported in several conditions characterized by a high incidence of osteoporosis such as rheumatoid arthritis (56, 57) and endometriosis (58, 59). In previous studies, we have shown that high IL-1 activity in vitro is characteristic of patients with high-turnover osteoporosis (20) and of early postmenopausal women, in whom bone remodeling is typically vigorous (21). Moreover, elaboration of IL-1 from PBMC has been found to be regulated (21, 60, 61) by estrogen and progesterone—substances well known for their bone-sparing effect. However, the mechanism(s) accounting for the release of IL-1 in states of high bone turnover remains to be elucidated. The results of this study suggest that collagen, hydroxyapatite, and possibly TGF-β enhance the capacity of PBMC to release IL-1. Since these factors are mobilized during bone resorption (23, 62), and/or constitutively secreted by osteoblasts (63), IL-1 could be secreted during physiologic bone remodeling in specific sites upon activation of new remodeling units. Because of the autocrine effect of IL-1 (64) and its powerful activity as a bone resorber (7–11), it is possible that IL-1 could initiate a cascade of events that leads to a further production of IL-1 and to an amplification of bone resorption. In this regard, it is noteworthy that the largest increase in IL-1 activity was induced by collagen fragments, substances more likely than intact collagen to be released locally during bone remodeling. Should this be the case, bone turnover could be regulated both by the amounts of matrix fragments released locally and by the degree of PBMC responsiveness to these fragments. The latter type of regulation is suggested by the ability of ovarian steroids and 1,25(OH)2D3 to regulate PBMC IL-1 activity (21, 60, 61) and the expression of collagen receptors on monocytic cells (65), respectively. In this model, the increase in bone turnover and IL-1 observed after the menopause could be visualized as the result of an enhanced responsiveness of mononuclear cells to the release of bone matrix fragments. Our findings, however, could also suggest that bone resorption is controlled by an IL-1 independent mechanism; the enhanced IL-1 activity observed in states of high bone turnover could be regarded as a consequence, rather than a cause, of increased bone resorption. Although this possibility cannot be discounted, it is unlikely that the substantial release of IL-1 that follows the adherence of mononuclear cells to bone surfaces would have no influence on bone resorption.

The accumulated data support the hypothesis of a major role for IL-1 in bone remodeling and provide a mechanism to explain, at least in part, the finding of increased IL-1 activity in patients with high bone turnover.

Acknowledgments

We thank Ms. Bernice Kaplan and Mr. James Havranek for secretarial and editorial assistance.

Dr. Malone supported by a “Merit Review” grant from the Veterans Administration. Dr. Santoro is an Established Investigator of the American Heart Association. This study was supported in part by grants from the National Osteoporosis Foundation and the National Institutes of Health (AR-39706).

References


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